Article Summary Line:

The parthenogenetic population of Asian longhorned tick is broadly distributed in China and plays a major role in the long-distance spread of SFTSV and perhaps future invasion of countries outside of Asia.

Running Title: Rapid spread of SFTSV

Keywords: Severe Fever with Thrombocytopenia Syndrome, tick, parthenogenesis, spread.

Severe fever with thrombocytopenia syndrome virus and parthenogenetic Asian longhorned tick *Haemaphysalis longicornis* (Acari: Ixodidae)

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Abstract

Severe fever with thrombocytopenia syndrome virus (SFTSV) is spreading rapidly in Asia. It is transmitted by *Haemaphysalis longicornis* (Asian longhorned tick, ALT), which has both parthenogenetically and sexually reproducing populations. Parthenogenetic populations were found in at least 15 provinces in China and strongly correlated with the distribution of SFTS. The distribution of SFTS cases was however poorly correlated with the distribution of populations of bisexual ALTs. Phylogeographic analysis suggested that the parthenogenetic population spread much faster than bisexual population because colonization is independent of sexual reproduction. A higher proportion of parthenogenetic ALTs were collected from migratory birds captured at an SFTS endemic area, implicating the contribution to the long-range movement of ALTs in China. The SFTSV susceptibility of parthenogenetic females was like that of bisexual females under laboratory conditions. These results suggest that parthenogenetic ALT, probably transported by migratory birds, play a major role in the rapid spread of SFTSV.

Text

Introduction

Parthenogenesis is the development of an embryo from an unfertilized egg and is a common reproductive mechanism in invertebrate arthropods, especially insects and mites (1). One of the potential advantages of parthenogenesis is that the offspring are all genetically identical, and in relatively stable environments this can lead to a rapid expansion in numbers. Furthermore, following dispersal, new populations can be established from a single female. The Asian long-horned tick (*Haemaphysalis longicornis* Neumann, 1901; ALT) is one of a small number of medically important tick species that have both parthenogenetic (parth-) and bisexual (bi-)

80 populations (2). The parth- population of ALT originated in Northern Japan (3) and is now
81 common in the Asia-Pacific region. Both parth- and bi- populations occur in East Asia, but only
82 the parth- population is found in Oceania (3). In China, the parth- population has only been
83 reported in a few locations (4). In 2017, parth- ALT were found in New Jersey, USA (5) and by
84 2020, had been found across 12 states primarily within the Eastern USA (6).
85 Severe fever with thrombocytopenia syndrome virus (SFTSV) is a tick-borne phlebovirus
86 transmitted by ALT and was first described in China in 2009 at the border of Henan, Anhui, and
87 Hubei provinces (7, 8). SFTSV is maintained and transmitted by ALTs in the larva, nymph, and
88 adult stages in both transovarial and transstadial modes (8-10). Human mortality rates resulting
89 from SFTSV infection range from 6% to 30% (7, 11). From 2011 to 2016, SFTS cases were
90 reported in 18 of the 34 provinces in China, with a 3-fold increase in the case number (from 500
91 cases to 1500 cases) (11). Most cases were in the rural areas of Henan (37%), Shandong (26.6%),
92 Anhui (14%), and Hubei (12.6%) (11). SFTSV has also been reported in Republic of Korea (2012),
93 Japan (2014), Vietnam (2019), and Pakistan (2020) (12-15). Phylogenetic analysis revealed that
94 SFTSV isolates separate into the Chinese clade and the Japanese clade, which is consistent with
95 their geographic distribution (16). A close relative of SFTSV, Heartland virus, was reported in the
96 USA in 2012 transmitted by *Amblyomma americanum* (17, 18).

97 ALT is the dominant tick species in the SFTSV endemic area, with proportional representation in
98 the tick fauna of 88% in Jiangsu Province, China, and 91% in Gangwon Province, Republic of
99 Korea (19, 20). Most of the SFTSV endemic areas are rural, and 97% of the patients are farmers
100 living in wooded and hilly areas, far from modern transportation and cities (21). The rapid spread
101 of SFTSV is currently unexplained, although ALT has a broad host range (18), enabling several
102 possible modes of dissemination. For example, livestock and wild mammals are common hosts for

ALT so the grazing movement of cattle or foraging of wild mammals, such as hares, could rapidly distribute ticks in an area with a suitable habitat (22). Furthermore, birds range far enough daily to transport ticks within a district, while long-range dispersal of tick-borne pathogens can also be accelerated by tick-infested migratory birds (23). In 2016, SFTSV antibodies were detected in tick-infested migratory Greater White-fronted Geese in Jiangsu Province, China (24). This finding has led to the suggestion that migratory birds may have been involved in the rapid spread of this disease within China. Two areas of China that have high endemic levels of SFTSV are both situated on major bird migratory routes (25). The Dabie Mountain area region (where SFTSV first reported), is in the middle of an important bird migration route from Dongting Lake and Poyang Lake to Siberia, while Penglai City and Dalian City are both located on the northern part of the Asia-Pacific migratory route. Dongting Lake and Poyang Lake are two of the most important overwintering sites for migratory birds in China (26, 27).

The aim of this study was to test the hypothesis that parth- ALT, possibly carried by migratory birds, are responsible for the rapid spread of SFTSV in China and Asia. To test this hypothesis we carried out a series of linked experiments, which included mapping the distribution of bi- and parth- ALTs in those Chinese provinces with a high prevalence of SFTSV, estimating the geographical correlation between bi- and parth- ticks and SFTSV cases for those provinces, surveying the infestation of ALT in migratory birds in a high prevalence SFTSV city, testing the virus acquisition and transstadial passage of ALTs for SFTSV, inferring the phylogeny of ALTs using ticks collected from around the world, and estimating the correlation between migratory bird routes and bi- and parth- ticks populations within China.

Materials and Methods

Tick Collection in China

126 ALTs were collected from 73 counties covering 20 provinces where SFTSV is endemic (Fig. 1A
127 and S2). Ticks of all life stages were collected by flag-dragging and removal directly from
128 animals between April and November 2019. Ticks were identified based on morphological
129 characteristics, visualized through a light microscope, with further molecular confirmation in the
130 laboratory by sequencing the mitochondrial 16S ribosomal RNA (16S rRNA) gene. Primers:
131 (16S-1) CTGCTCAATGATTTTTTAAATTGCTGTGG and (16S-2)
132 CGCTGTTATCCCTAGAGTATT. A single leg was removed from each tick for the molecular
133 analysis to confirm identification. A random sample of 5 or 6 live tick from each county sampled
134 were then stored at room temperature until ploidy detection, whilst the remaining tick specimens
135 were stored at -80℃.

136 **Tick collection overseas**

137 Eight extracted tick DNA samples were collected from overseas collaborators including Japan,
138 Republic of Korea, New Zealand, Australia, and the USA in 2019 and subjected to the same
139 molecular analysis as the Chinese collected ALT ticks.

140 **Polyploid analysis of ticks**

141 Bi- and parth- ticks are difficult to distinguish using classical taxonomic methods, but they can be
142 identified by karyotype analysis, so flow cytometry was used to test the ploidy of the tick
143 chromosomes (4). This was accomplished by measuring the fluorescence intensity of cell nuclei
144 stained with DAPI (4′,6-diamidino-2-phenylindole). Sysmex Partec CyFlow Space (Sysmex
145 Partec, Görlitz, Germany) was used in this analysis and Sysmex Partec CyStain DNA 1 Step kit
146 was used.

147 **Correlation between bi- and parth- ALTs and SFTS cases**

148 The geographical correlation between SFTS cases and the distribution of different populations of
149 ALT was analyzed by linear regression (28) . The total number of recovered ticks, aggregated at
150 the province or municipality level, was used as the independent variable, and the incidence of
151 SFTS cases (cases per million population) reported in each respective province or municipality
152 was used as the dependent variable. SFTS cases data summarized by province or municipality
153 for the year 2019, were obtained from the Chinese Center for Disease Control and Prevention.

154 **Phylogenetic tree and genetic distance**

155 For each county, one bi- tick sample or/and one parth- tick sample were picked randomly for whole
156 mitochondrial sequencing. The whole mitochondrial genomes were sequenced by next-generation
157 sequencing (Tsingke, Beijing, China). Phylogenetic analysis was performed using the whole
158 mitochondrial genomes of 46 bi- and 35 parth- ticks collected from China, Japan, Republic of
159 Korea, New Zealand, Australia, and the USA. Tick DNA was extracted using the MightyPrep
160 reagent for DNA Kit (Takara, Japan) according to the manufacturer's instructions. The
161 mitochondrial DNA were sequenced by next generation sequencing (Tsingke Biotech, Beijing,
162 China) and deposited in GenBank (Accession number MW642336-MW642407). Two
163 phylogenetic trees were constructed using this data, the first using the maximum likelihood method,
164 MEGA-X with the bootstrap value set at 1000 and the second using the Bayesian interference
165 method, MrBays-3.2.7 (http://nbisweden.github.io/MrBayes/index.html) performing 1,500,000
166 generations. The genetic distance (GD) used to calculate the dispersal index was equal to the
167 nucleotide substitution rate.

168 **Nucleotide diversity**

169 The nucleotide diversity (Pi) in each dataset is estimated by:

170 $$Pi = 2\sum_{i<j} d_{ij}/[n(n-1)], \quad (1)$$

171 where d_{ij} is an estimate of the number of nucleotide substitutions per site between DNA
172 sequences i and j and n is the number of DNA sequences examined (29). The calculation for Pi
173 was implemented in DnaSP-V6 (30).

174 **Dispersal index**

175 Without longitudinal surveillance data, we could not evaluate the spread velocity of ticks directly,
176 so we defined a dispersal index to quantify and compare the dispersal ability of bi- and parth- ticks.
177 According to the molecular clock theory, the mutation rate is relatively constant, so genetic
178 distance can be used to represent time distance (31). The dispersal index (I) was defined as: I=D/G.
179 Where, D represents the sum of the spatial distances between every two samples (i.e. pairwise
180 geographical distance), and G represents the genetic distance (nucleotide substitutions) between
181 every two samples (i.e. pairwise genetic distance). This index is not the true dispersal velocity
182 itself, but we can infer that the greater the dispersal index value then the greater the dispersal
183 velocity must be. An independent sample t-test was performed to contrast the dispersal rates for
184 bi- and parth- ticks. The calculation of dispersal index and t-test were implemented by customed
185 scripts written in Python-3.7 (https://www.python.org).

186 **Migratory bird capture and tick collection.**

187 To test our hypothesis that migratory birds are carrying ALT ticks, we investigated the presence
188 of ticks on northward migratory birds at a high-density stopover site at Penglai (37°55′13.84″N,

189 120°43′53.27″E), Yantai City, Shandong Province in April 2021. Shandong Province is an
190 important location on the Asia-Pacific migratory route. Birds were captured with mist nets (2.5 x
191 6 m or 2.5 x 12 m, mesh size：3.0 cm) placed in a wooded habitat. Upon capture, birds were
192 meticulously examined for the presence of ticks by searching the ear canals, back of the head,
193 mandibular area, and perimeter of the eyes. Ticks were then removed with fine forceps.

194 **Penglai City tick diversity**

195 Penglai City is one of the most endemic areas of SFTS in China and an important location in the
196 Asia-Pacific migratory route. Parth- ticks were collected and sequenced from nine locations in
197 Penglai City. Phylogenetic analysis was used to compare the diversity of ticks from Penglai city
198 to 15 other provinces in China.

199 **Virus acquisition and transstadial passage of ALTs for SFTSV**

200 The virus susceptibility of parth- and bi- populations for transmitting SFTSV was tested using
201 laboratory-adapted ALT colonies and an interferon α/β receptor knockout ($IFNAR^{-/-}$) mouse
202 model. Nymphal ALTs were infected by feeding on $IFNAR^{-/-}$ C57/BL6 mice, previously
203 inoculated with 2×10^3 FFU of SFTSV (Wuhan strain; GenBank accession numbers: S,
204 KU361341.1; M, KU361342.1; L, KU361343.1). The fed nymphs were collected after they were
205 fully engorged and had detached from the mice. SFTSV RNA levels in the ticks were analyzed
206 after they molted into adults. Total RNA prepared from the homogenates of the ticks was extracted
207 using TRIzol reagent (Thermo Fisher Scientific, USA) according to manufacturer instructions.
208 Samples were analyzed using a One-Step SYBR PrimerScript reverse transcription (RT)-PCR kit
209 (TaKaRa, Japan) on an Applied Biosystems QuantStudio. Each sample was measured in triplicate.
210 The primers were designed as previously described [32].

211 # Results

212 ## Tick distribution and ploidy analysis

213 There were 1328 ALTs confirmed by 16S rRNA sequencing, of which 271 (20.4%) live ticks were
214 further submitted for ploidy analysis by flow cytometry (255 ticks) or by mitochondrial sequencing
215 (16 ticks) (Table S1). Ploidy testing showed a peak for bi- (diploid) population at the 66 position
216 and for the parth- (triploid) population at the 99 position (Fig. 1A). Of the ticks submitted for
217 ploidy analysis, 186/271 (69%) were identified as bi- and 85/271 (31%) were identified as parth-.
218 Bi- ticks were detected in 55/73 (75%) of counties, parth- ticks were detected in 30/73 (41%) of
219 counties, and a mixture of both populations were detected in 12/73 (16%) counties (Fig. 1B and
220 Table S1). Interestingly in 18/73 (25%) of counties only parth- ticks were found and in 43/73 (59%)
221 of counties only bi- ticks were found.

222 ## Correlation of SFTSV with bi- and parth- populations of ALTs

223 SFTSV cases showed a strong correlation with the parth- population ($R^2 = 0.685$, $p < 0.001$), but
224 almost no correlation with the bi- population ($R^2 = 0.026$, $p = 0.501$) (Fig. 2). In the highly endemic
225 Dabie mountain area (located at the border of Henan, Anhui, and Hubei provinces in central China),
226 11 out of 14 counties 66% of the collected ALTs were parth- (Table S1). These results suggest that
227 the parth- populations of ALT are geographically strongly associated with cases of SFTSV.

228 ## Phylogenetic analysis of bi- and parth- populations

229 For each county, one bi- or/and parth- ALTs were submitted for mitochondrial sequencing. Eighty
230 one whole mitochondrial genomes were obtained from 73 Chinese ticks and 8 ticks outside of
231 China. Fig. 3A, Fig. S3 and Fig. S4 clearly show that the parth- and bi- populations divide into
232 two distinct lineages that can be discriminated by a single T deletion at nucleotide 8497 in the un-

233 translational region (Fig. S5). This suggests that the parth- population may have originated from a
234 single event without gene exchange. The mean genetic distance (GD) between all sequences was
235 0.0078 as measured by nucleotide substitution rate. The New Zealand and Australian parth- strains
236 were very similar to the parth- strain from Okayama, Japan (Mean GD = 0.0003). The parth- strain
237 from Kagoshima, Japan, was a close relative to strains collected from Beijing, Hubei, and Henan
238 (Fig. S1A ⑧④②), which are geographically separate. The strain from New Jersey, USA, was
239 very similar to the strain from Jeju Island, Republic of Korea (GD = 0.0001).

240 **Genetic diversity**

241 Despite the loss of sexual reproduction, high genetic diversity has been reported in the asexual
242 populations of many different species (32). The genetic diversity (Pi) of the bi- and parth-
243 population, as measured by the mitochondrial genome, was 0.00249 and 0.00188, respectively.
244 These results indicate that the genetic diversity of the bi- and parth- population was similar and
245 that the parth- population may have diverged from the bi- population at a very early age.

246 **Dispersal index of bi- and parth- ticks**

247 Compared with bi- ticks, parth- ticks have a wider pairwise geographical distance distribution and
248 a narrower pairwise genetic distance distribution (Fig. 3B). The dispersal index of parth- ticks was
249 significantly higher than bi- ($t = 7.67$, $p < 0.001$), with the mean dispersal index for parth- ticks
250 (910228) being 2.3 times higher than for bi- ticks (393156) (Fig. 3C, D), indicating that parth-
251 ticks have a higher dispersal capacity.

252 **The correlation between migratory birds and ALTs**

253 Migratory birds were collected and examined for ALTs at Penglai City, which is a high SFTSV
254 endemic area and located in the Asia-Pacific migratory route (Fig.S1 ⑩). Ninety-five birds

255 belonging to 17 species were netted however, ALTs were only found on four species (n=54);
256 Naumann's thrush (*Turdus naumanni),* the Grey-backed Thrush (*Turdus hortulorum),* the great tit
257 *(Parus major)*, and the chestnut-eared bunting (*Emberiza fucata).* Only a total of 27 ticks were
258 recovered from these birds of which 19/27 (70%) were identified as ALT, with 17/19 (89%) ALTs
259 being parth- (Table 1). All recovered ALTs were nymphs.

260 **Penglai city tick diversity**

261 Phylogenetic analysis showed that the mitochondrial sequences of the parth- ALTs collected in
262 Penglai city from vegetation were highly diverse when aligned with those from 15 provinces in
263 China (Fig. S6). These data suggest that ticks of many different provinces were present in Penglai
264 city and likely spread there by migratory birds.

265 **Virus acquisition and transstadial passage of ALTs for spreading SFTSV**

266 A robust viremia was detected in the mice inoculated with SFTSV (Fig. 4A). After feeding until
267 engorgement and moulting, both the parth- and bi- population developed average titers of 3 log
268 RNA copies/mg without obvious differences (Fig. 4B). The SFTSV-acquisition and transstadial
269 passage efficiency of the parth- population appeared comparable to that of the bi- population.

270 Discussion

271 We found that the parth- population of ALT is more widely distributed in China than previously
272 thought and that the distribution is highly correlated with the endemic regions of SFTSV.
273 Phylogeographic analysis suggests that the parth- ALT population has spread more rapidly over a
274 greater distance than the bi- population and assessment of virus acquisition and transstadial
275 passage showed that bi- and parth- populations were comparable in maintaining the local
276 transmission of SFTSV. Although only a small number of ticks were recovered, parth- ALTs were
277 found dominant in migratory birds collected at a SFTS endemic area. We suggest that together

these results strongly support the hypothesis that parth- ALT populations are responsible for the rapid spread of SFTSV within China, most likely through being disseminated by migratory birds.

If, as we suggest, migratory birds have played an important role in the spread of parth- ALT then this would partly explain the wide distribution of these ticks from the cold Far East of Russia to the tropical areas of Australia and the Fiji Islands. However, the important role of livestock, wild mammals, and humans via companion animals in the translocation of parth- ALTs should not be overlooked (22).

Migratory birds have long been known to be carriers of ticks. Penglai City is one of the most endemic areas of SFTS and is a key passage in the northern part of East Asian-Australasian Flyway. In this area, 96% of ALTs were parth- and showed extremely high diversity (Fig. S6). During a spring bird survey in Penglai City in 2021, ALTs were specifically found in four bird species *Turdus naumanni, Turdus hortulorum, Parus major*, and *Emberiza fucata* and 89% of them were parth-. Among the four bird species, *Turdus naumanni, Turdus hortulorum,* and *Emberiza fucata* migrate between East Asia and Siberia, and are occasionally found in Alaska (www.ebird.org). The preferred habitats for these 4 species are grasslands and bushes, which are also the preferred habitats of ALTs. These results suggest that migratory birds have an important role in the long-range movement of parth- ticks within China and potentially even trans-oceanic spread of SFTSV.

Parth- ALTs are also implicated in the spread of a pathogenic form of the blood parasite, *Theileria orientalis* throughout the Asia-Pacific (18). ALTs are purported to have been introduced to Australia in the 19th century from Northern Japan and later disseminated to New Zealand, New Caledonia, and Fiji. This theory is supported by phylogenetic results of this study which show that the New Zealand and Australian ALTs are alike and closely resemble the parth- strain from

301 Okayama, Japan (33). *T. orientalis* has been present in Australia for more than 100 years, having
302 been introduced with the vector tick, and until 2006 caused only minor symptoms in livestock (34).
303 In 2006, the pathogenic Ikeda genotype of *T. orientalis* was introduced from Eastern Asia into
304 New South Wales, Australia (35) and by 2014, had spread to most of the states (36). The recent
305 spread of *T. orientalis* across the Asia-Pacific and into North America highlights the risk of rapid
306 disease agent transmission into areas where a competent vector (ALT) is already established. Thus,
307 while SFTSV has not yet been detected in the Western Hemisphere, the presence of ALT parth-
308 ticks in several countries within this region presents a clear risk for the emergence of this disease
309 in the future.

310 **Ethics statement**

311 All animal studies were carried out in accordance with the recommendations in the Guide for the
312 Care and Use of Laboratory Animals of the Ministry of Science and Technology of the People's
313 Republic of China. The protocols for animal studies were approved by the Committee on the Ethics
314 of Animal Experiments of the Institute of Zoology, Chinese Academy of Sciences (Approval
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409

410 Table 1. *Haemaphysalis longicornis* tick (ALT) collected from migratory birds and their hosts in

411 Penglai City 2021.

Avian host	**No. of examined birds**	**No. of birds with ticks**	**No. of ticks**	**No. of ALT**	**Percent of Parth- (%)**
Turdus naumanni	45	8	11	3	33
Turdus hortulorum	7	2	8	8	100
Parus major	1	1	5	5	100
Emberiza fucata	1	1	3	3	100

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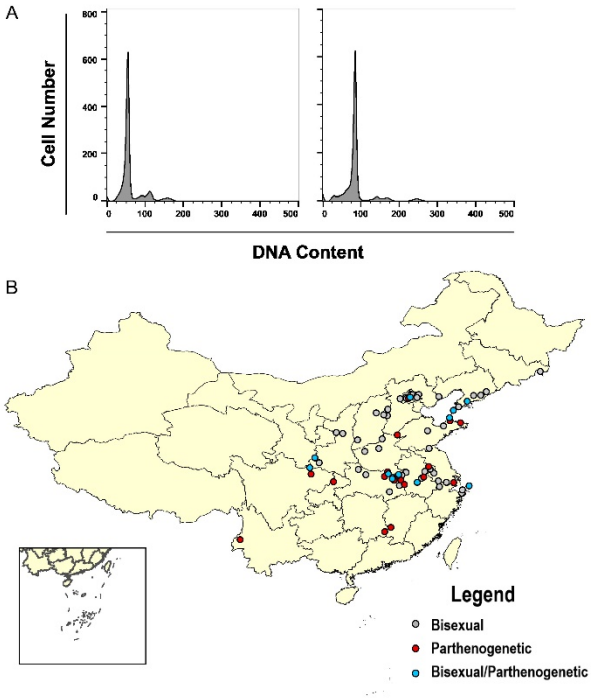


413

414 **Figure 1. Polyploid analysis and geographical distribution of bi- and parth- Asian**

415 **longhorned tick (ALT) populations.** (A) The ploidy of ticks ALTs was tested using flow

416 cytometry by measuring the fluorescence intensity of cell nucleus stained with DAPI. Illustrated

417 is a representative of bi- (2n) with peak at the 66 position and parth- (3n) at 99. (B) Geographical

418 distribution of bisexual and parthenogenetic ALTs collected in China. Red dots indicate parth-;

419 gray dots indicate bi-; blue dots indicate both bi- and parth-.

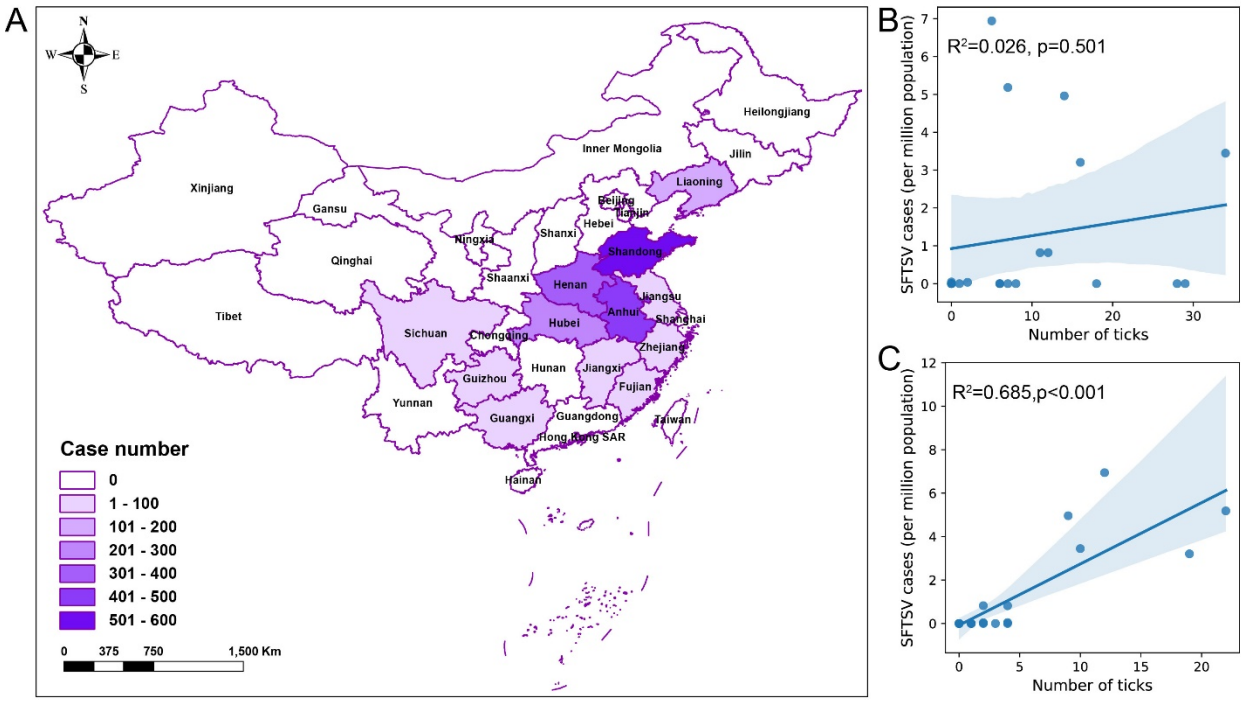
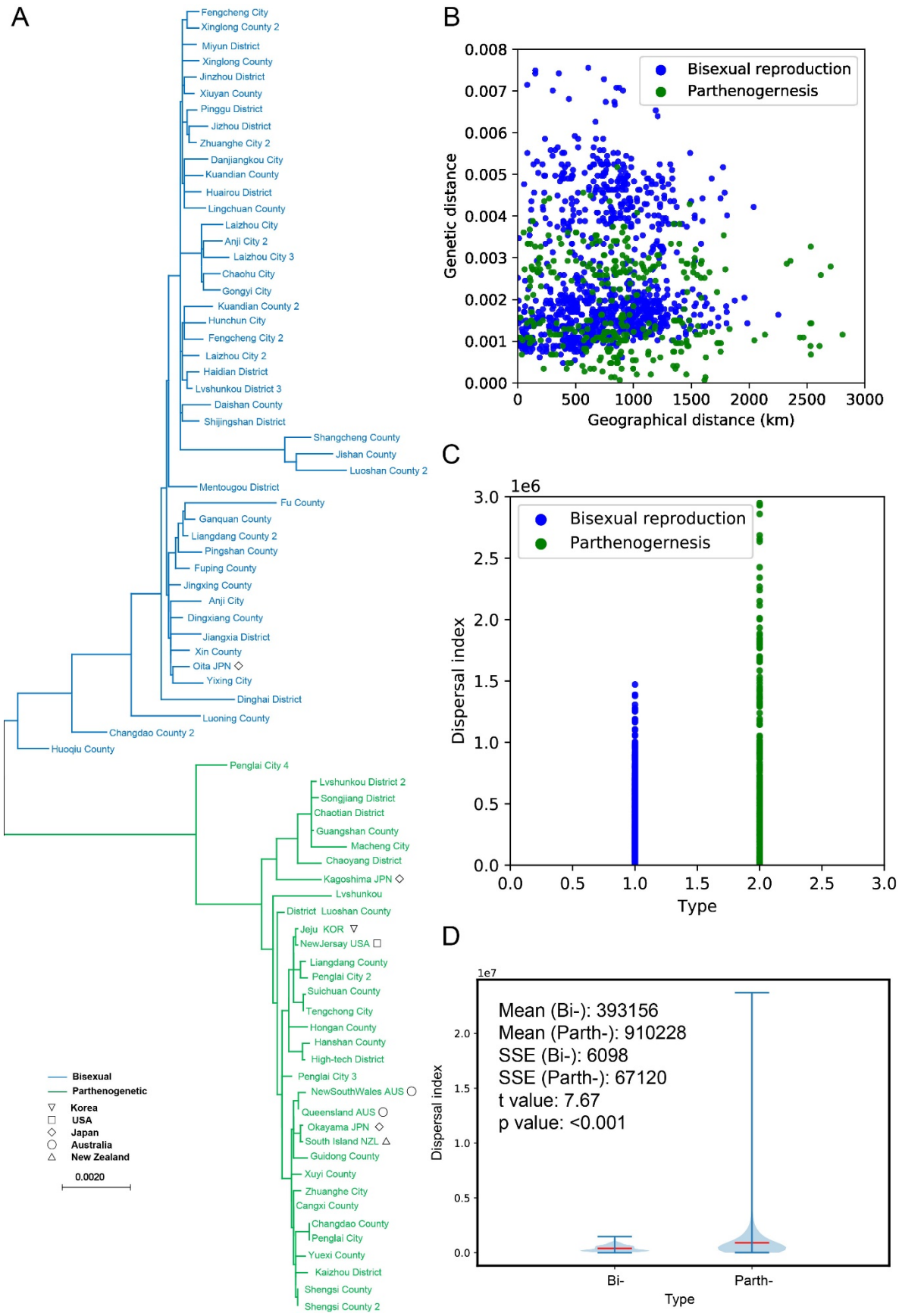
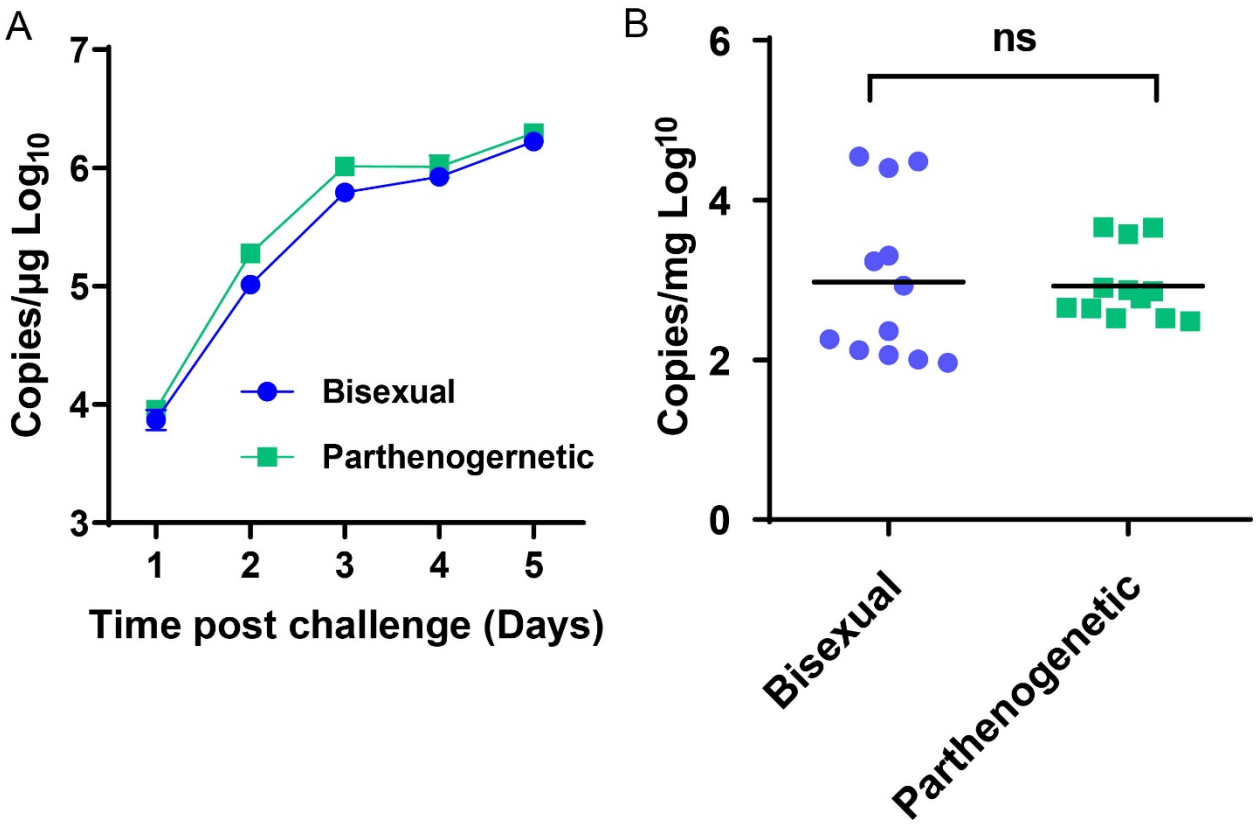


Figure 2. The SFTS endemic area is highly correlated with the parth- Asian longhorned tick (ALT) population. (A) The distribution of Severe fever with thrombocytopenia syndrome (SFTS) cases in China in 2019 (China CDC). B and C show that the correlation between incidence of SFTS cases (cases per million population) and the number of bi- population (B) and the parth- population (C) ALTs in different provinces. Each dot represents a province.


A
Fengcheng City
Xinglong County 2
Miyun District
Xinglong County
Jinzhou District
Xiuyan County
Pinggu District
Jizhou District
Zhuanghe City 2
Danjiangkou City
Kuandian County
Huairou District
Lingchuan County
Laizhou City
Anji City 2
Laizhou City 3
Chaohu City
Gongyi City
Kuandian County 2
Hunchun City
Fengcheng City 2
Laizhou City 2
Haidian District
Lvshunkou District 3
Daishan County
Shijingshan District
Shangcheng County
Jishan County
Luoshan County 2
Mentougou District
Fu County
Ganquan County
Liangdang County 2
Pingshan County
Fuping County
Jingxing County
Anji City
Dingxiang County
Jiangxia District
Xin County
Oita JPN
Yixing City
Dinghai District
Luoning County
Changdao County 2
Huoqiu County
Penglai City 4
Lvshunkou District 2
Songjiang District
Chaotian District
Guangshan County
Macheng City
Chaoyang District
Kagoshima JPN
Lvshunkou
District Luoshan County
Jeju KOR
NewJersay USA
Liangdang County
Penglai City 2
Suichuan County
Tengchong City
Hongan County
Hanshan County
High-tech District
Penglai City 3
NewSouthWales AUS
Queensland AUS
Okayama JPN
South Island NZL
Guidong County
Xuyi County
Zhuanghe City
Cangxi County
Changdao County
Penglai City
Yuexi County
Kaizhou District
Shengsi County
Shengsi County 2
Bisexual
Parthenogenetic
Korea
USA
Japan
Australia
New Zealand
0.0020
B
Bisexual reproduction
Parthenogernesis
Genetic distance
Geographical distance (km)
C
1e6
Bisexual reproduction
Parthenogernesis
Dispersal index
Type
D
1e7
Mean (Bi-): 393156
Mean (Parth-): 910228
SSE (Bi-): 6098
SSE (Parth-): 67120
t value: 7.67
p value: <0.001
Dispersal index
Bi-
Parth-
Type

426

427 **Figure 3. Phylogenetic and phylogeographic analysis of the bi- and parth- Asian**
428 **longhorned ticks (ALTs).** (A) Maximum likelihood tree established with the mitochondrial
429 genomes of ALTs collected in the Asian-Pacific region. Multiple ALTs from the same county
430 were marked by number. (B) Distribution of bi- and parth- ALTs in pairwise genetic distance and
431 pairwise geographical distance. (C) Distribution and (D) the differences of dispersal index (see
432 methods) between bi- and parth- ALTs. The red bar in the violin plot represents the mean
433 dispersal index, and the shaded blue areas indicate the kernel density estimation of the dispersal
434 indices.



435

436 **Figure 4. Susceptibility of bi- and parth- Asian longhorned ticks (ALTs) to Severe fever**
437 **with thrombocytopenia syndrome virus (SFTSV).** Groups of bi- or parth- nymph ALTs were
438 fed separately on one IFNAR$^{-/-}$ C57/BL6 mouse that was intraperitoneal inoculated with $2x10^3$

439 FFU of SFTSV. (A) Viremia of IFNAR$^{-/-}$ C57/BL6 mice were monitored by Real-time PCR

440 during tick feeding. (B) SFTSV infection in the ALTs were tested by Real-time PCR after

441 molting into adult. Each dot represents one tick.

442